Research paper

Correspondence analysis between traditional Chinese medicine (TCM) syndrome differentiation and histopathology in colorectal cancer

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A B S T R A C T

Introduction: Defining Traditional Chinese medicine (TCM) syndrome is considered the key therapeutic principle of TCM. The present study was to explore the correlation between TCM syndrome differentiation and histopathology in colorectal cancer (CRC).

Methods: A total of 180 patients were differentiated into 5 TCM syndrome types, including accumulated damp-heat type (ADH), deficiency of both qi and blood type (DBQB), deficiency of liver and kidney yin type (DLKY), deficiency of spleen and kidney yang type (DSKY) and qi stagnation due to spleen deficiency type (QSSD). They were also differentiated into pathologic types, including adenocarcinoma, mucous carcinoma, signet ring cell carcinoma and anaplastic carcinoma. Moreover, the expression of protein 53 (p53), cluster of differentiation 44 (CD44), non-metastasis 23 (nm23), proliferating cell nuclear antigen (PCNA) and B-cell lymphoma-2 (Bcl-2) was detected by immunohistochemistry in 61 patients. Finally, the correspondence analysis between TCM syndrome differentiation and histopathology or pathological molecular markers was conducted.

Results: Chi-square test of independence showed that TCM syndrome types correlated with pathological types ($\chi^2 = 33.456, P = 0.001$), while not with molecular markers ($\chi^2 = 7.344, P = 0.834$). Correspondence analysis showed that the distances between adenocarcinoma and QSSD or DLKY were the shortest among the distances of adenocarcinoma with other TCM syndrome types. Moreover, distances between mucinous carcinoma and ADH, signet ring cell carcinoma and ADH, anaplastic carcinoma and DBQB were the shortest among all the types.

Conclusion: TCM syndrome differentiation is strongly correlated with histopathology of colorectal cancer. TCM syndrome differentiation may be used as a supplement in the diagnosis of CRC.

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1. Introduction

The development of colorectal cancer (CRC) is thought to be due to uncontrolled cell growth in the rectum, colon, or appendix. It ranks the first in incidence among malignancies and the second in cancer-related deaths in Europe for both genders [1,2]. There are 446,000 new cases of CRC occurring annually in Europe which means that it is a major health issue [3]. The five-year survival rate for CRC is less than 60% in Europe [4]. In addition, more than 90% of CRC s have metastasized or advanced by the time they are diagnosed [5]. Furthermore, the treatments for CRC are always accompanied by side effects including fecal incontinence, sexual dysfunction and bowel dysfunction [6]. Therefore, exploring new tactics for the treatment of CRC is necessary.

Traditional Chinese medicine (TCM) with its holistic emphasis and the connection of man with the social and natural environment has been used to treat CRC over the last 6000 years with some degree of success [7]. Nowadays, TCM is commonly used for
alleviating the side-effects of surgery and standard treatment such as chemotherapy or radiotherapy, and for improving patient quality of life [8]. TCM has benefits in its own right, which has been reported to induce rectal cancer cell apoptosis [9], prevent tumor metastasis [10], and directly alleviate patients’ symptoms such as bleeding, diarrhea, pain, nausea and vomiting [11–13]. TCM may also have direct anti-cancer effects, as described previously [14]. Moreover TCM therapy which could provide a theoretical and practical approach for treating CRC is characterized by treatment based on “syndrome differentiation”. Since individual tumors exhibit different pathologic features, specific therapy for CRC based on their pathologic features, this is the ultimate goal of treatment.

The traditional Chinese medicine (TCM) is the essence of Chinese culture. Syndrome differentiation is a key principle in TCM [15]. As described previously, clinical treatment of a patient is based on the successful differentiation of a specific syndrome [16]. TCM syndrome differentiation has been widely used in diagnosis of diseases including infection, inflammation and autoimmune disorders [15]. Moreover, it bears a close relationship with pathological characteristics in diseases, such as IgA nephropathy [17], non-small cell lung cancer [18] and chronic gastritis [19]. Furthermore, TCM syndrome differentiation and treatment can improve the prognosis of CRC in elderly patients [20]. Yin deficiency and qi deficiency syndrome are considered to be the factors for poor prognosis of CRC [21]. However, study focusing on the relationship between TCM syndrome typing and pathological typing in CRC is rare.

In this study, primary research was carried out on the correlation of TCM syndrome type and pathological characterization of patients with CRC. Patients were differentiated into 5 syndromes. Meanwhile, they were also differentiated into 4 kinds of CRC according to pathological characterization. Moreover, some of the patients were also diagnosed by the pathological molecular markers, including protein 53 (p53), cluster of differentiation 44 (CD44), non-metastasis 23 (nm23), proliferating cell nuclear antigen (PCNA) and B-cell lymphoma-2 (Bcl-2). Finally, the correspondence analyses between TCM syndrome types and pathological types or pathological molecular markers were conducted. This research reveals the feasibility of TCM syndrome differentiation in diagnosis of CRC.

2. Materials and methods

2.1. Patients

The study took place in Shu Guang hospital affiliated to Shanghai University of traditional Chinese medicine between March 2005 and January 2009. A total of 180 patients (approved by the ethical committee of Shanghai TCM-integrated Hospital, Shanghai University of TCM) with confirmed CRC were enrolled in this study. The average age was 62 ± 12 years (range, 31–85 years). These patients included 98 males (54.4%) and 82 females (45.6%). Of these, 107 cases (59.4%) had a diagnosis of colon cancer and 73 (40.6%) had a rectal cancer diagnosis.

The sample size was calculated to provide 80% power with the chi-square value of 4.2 after testing the correlation between the TCM syndrome types and pathological types using chi-square test of independence, with a two-sided 5% significance allowing for an anticipated dropout rate of 10%.

2.2. Inclusion and exclusion criteria

Patients were included if they were cytologically or pathologically diagnosed with CRC [22]. In addition, patients diagnoses had to be confirmed by a TCM physician to ensure that patients exhibited one of the five TCM syndromes (showed in TCM syndrome differentiation) [23]. Patients were excluded for the following reasons: (1) Patients did not conform to the inclusion criteria. (2) Patients were treated with immunosuppressants or had serious diseases such as; heart, liver, kidney disease or conditions associated with the blood system. (3) Patients who were pregnant or had an infection were also excluded from our research. (4) Patients who stopped the treatment before the end of the treatment period or who did not comply with the doctors in terms of failing to provide the required data were not included in this study.

2.3. TCM syndrome differentiation

The TCM syndrome differentiation for each patient was conducted by two expert TCM physicians in our hospital. The process for TCM syndrome diagnosis was performed independently by each TCM physician and both of them were blind to the type of cancer that each patient had. Any discrepancies of TCM syndrome differentiation between them were resolved by subsequent discussion.

According to routine diagnosis and treatment method of traditional Chinese medicine for disease in Shanghai (Shanghai shi zhong yi bing zheng zhen liao chang gui) [22], the syndrome types were identified as follows: (1) Accumulated damp-heat type (ADH): symptoms include paroxysmal abdominal pain, tenesmus, passing stool with pus and blood, burning pain in anus, or fever, chest distress, bitter taste, deep-colored urine, red tongue with yellow and greasy coating, slippery pulse of arteries and veins. (2) Deficiency of both qi and blood type (DBQB): symptoms include dull abdominal pain, loose stool, shortness of breath and weak, pale complexion, or rectoceles drop, pink tongue with white coating, deep and thready pulse. (3) Deficiency of liver and kidney yin type (DLKY): symptoms include dizziness, soreness of loins and tinnitus, low-grade fever and night sweating, dysphoria with feverish sensation in chest bitter taste and dry throat, constipation, red tongue with little or no coating, thread pulse. (4) Deficiency of spleen and kidney yang type (DSKY): symptoms include fear of cold and cold limbs, loose stool, frequent defecating or even diarrhea at dawn, continuous abdominal pain, soreness and weakness of waist and knees, pale complexion, short of breath and weakness, plump tongue with white, thin or greasy coating, thread, soft and weak pulse. (5) Qi stagnation due to spleen deficiency type (QSSD): symptoms include abdominal distension and anorexia, borborygmus and scurrying abdominal pain, loose stool or blood stool, languid, sallow complexion, pink tongue with white, thin or greasy coating, soft pulse. CRC syndrome could not be diagnosed by tongue inspection only and be confirmed when two or more than two symptoms occurred in this patient. If more than two syndrome symptoms occurred in one patient, the syndrome which got more symptoms was regarded as the main syndrome.

2.4. The pathologic type differentiation

According to standards for diagnosis and treatment of CRC (2010) [22], pathologic types were identified as follows: (1) Adenocarcinoma, whose tissue was papillary or glandular. (2) Mucous carcinoma, which contained quantity of mucous in cancer tissue. (3) Signet ring cell carcinoma: the cytoplasm was full of mucus. Nuclear was rounded or oval and tilted to the side of cell. The cancer cell was presented as a signet ring. (4) Anaplastic carcinoma: cancers were diffusely infiltrated and did not represent as glands. The cancer cells were small and irregularly or round shaped. It was obvious to see the heteromorphism of nucleus. If the cancer belonged to more than one pathologic type, the type with the highest proportion was regarded as the main type. Moreover, if the types had the same proportion, the cancer was identified as the
type that had the lowest degree of histological differentiation degree.

2.5. Immunohistochemistry

A total of 61 patients of the 180 CRC patients received pathological molecular markers detection by immunohistochemistry. As patients with DBQB were rare, the molecular markers detection was not conducted in this syndrome.

Embedded tissues were sliced into 5-μm sections and deparaffinized in xylene followed by ethanol and rehydrated in PBS (pH 7.4). Paraffin-embedded tissues were used for hematoxylin eosin staining and immunohistochemistry. Sections analyzed for protein expression (PS3, CD44, nm23, PCNA, Bcl-2) were boiled in 0.01 M citrate buffer for 15–20 min at 95 °C in (pH 6.0) for antigen retrieval. After these pretreatment procedures, all samples were incubated with a solution of 1% hydrogen peroxide in methanol for 10 min at room temperature to block endogenous peroxidase. Sections were then incubated for 10 min at room temperature in 50 μL normal nonimmune goat serum. The rabbit anti-human monoclonal antibodies (primary antibodies, 1:100, DAKO, Carpinteria, CA, USA) for PS3, CD44, nm23, PCNA and Bcl-2 were applied to the sections overnight at 4 °C. The next day the sections were rinsed 3 times in PBS and incubated for 10 min with the addition of peroxidase-conjugated secondary antibody (goat anti-rabbit, 1:50, Wuhan Boster Biological Technology Co., Ltd., Wuhan, China) for 1 h at room temperature. Then, the samples were incubated with 50 μL streptavidin–peroxidase (S–P) for another 10 min. The samples were visualized with 100 μL stable diaminobenzidine (DAKO, Carpinteria, California, USA) for 10 min and were counterstained with Gill’s 3 hematoxylin (Sigma Chemical Co., St. Louis, USA). After rinsing in PBS, the slides were dehydrated by ethanol and mounted with neutral balsam. The slides were checked by optical microscope (CH20BIMF2000, Olympus, Japan). Negative controls involved the same procedure by replacing primary antibody with PBS.

2.6. Statistical analysis

Correspondence analysis between TCM syndrome differentiation and pathological characterization or pathological molecular markers was conducted by SPSS16.0 (SPSS Inc., Chicago, IL, USA). Distance between the row variable category and the column variable category in the same orientation represented the correlation between the two variable categories. The shorter the distance was, the stronger the correlation existed [24].

3. Results

3.1. The distribution of CRC in TCM syndrome types and pathological types

As shown in Table 1, adenocarcinoma accounted for the highest proportion at 83.9% (151 cases), mucinous carcinoma at 12.2% (22 cases), signet ring cell carcinoma at 1.5% (3 cases), and anaplastic carcinoma at 1.5% (4 cases).

Patients with QSSD and DLKY accounted for high proportion at 42.8% and 32.8%, following with ADH (12.8%) and DSKY (10.0%). DBQB accounted for the lowest proportion at 1.6%. For adenocarcinoma, DBQB and DLKY accounted for the highest proportion at 66.1% (119 cases). ADH and DSKY accounted for 16.7% (30 cases). For mucinous carcinoma, ADH and QSSD had the highest proportion at 10% (18 cases). For signet ring cell carcinoma, each ADH, QSSD and DLKY had one case, accounting for 1.5% in total. For anaplastic carcinoma, 4 cases were evenly distributed between QSSD, DSKY, DLKY and DBQB.

3.2. The correspondence analysis of TCM syndrome type and pathological type

Chi-square test of independence showed that TCM syndrome type was correlated with the pathological type ($\chi^2=33.456$, $P=0.001$) and correspondence analysis of TCM syndrome type with pathological type could be conducted. The results were shown in Fig. 1. The distances between adenocarcinoma and QSSD or DLKY were shorter than other TCM syndrome types. For mucinous carcinoma, the shortest distance occurred between this cancer and ADH. For signet ring cell carcinoma and anaplastic carcinoma, the shortest distance existed in ADH and DBQB, respectively.

3.3. CRC detected by pathological molecular markers

There were 34 cases with positive expression of p53, 37 cases with positive expression of CD44, 44 cases with positive expression of pCNA, 41 cases with positive expression of Bcl-2 and 50 cases with positive expression of nm23 (Table 2). For all the molecular markers, most cases were enriched in QSSD type, which accounted for the highest proportion (67.6%, 59.5%, 70.5%, 70.7%, and 66.0%).

3.4. Correspondence analysis between TCM syndrome differentiation and pathological molecular markers

As shown in Fig. 2, the distances between TCM syndrome types and molecular markers were all long. There were no obvious correlations between TCM syndrome type and pathological molecular markers ($\chi^2=7.344$, $P=0.834$) according to the results of chi-square test of independence.

4. Discussion

CRC is the second leading cause of cancer-related deaths in European in both men and women [1]. The diagnosis and treatment of CRC have drawn more attention in the medical field. TCM, which has been used for thousands of years in China, is currently beneficial to patients in treating cancer [25]. TCM syndrome is considered the key therapeutical principle of TCM.

Table 1

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Adeno carcinoma</th>
<th>Mucous carcinoma</th>
<th>Signet ring cell carcinoma</th>
<th>Anaplastic carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH</td>
<td>14 (7.8%)</td>
<td>8 (4.4%)</td>
<td>1 (0.5%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>QSSD</td>
<td>65 (36.1%)</td>
<td>10 (5.6%)</td>
<td>1 (0.5%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>DSKY</td>
<td>16 (8.9%)</td>
<td>1 (0.5%)</td>
<td>0 (0)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>DLKY</td>
<td>54 (30.0%)</td>
<td>3 (1.7%)</td>
<td>1 (0.5%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>DBQB</td>
<td>2 (1.1%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

ADH: accumulated dampness-heat type; QSSD: qi stagnation due to spleen deficiency type; DSKY: deficiency of spleen and kidney yang type; DLKY: deficiency of liver and kidney yin type; DBQB: deficiency of both qi and blood type.
In this study, we investigated the relationship between TCM syndrome types and pathological types or pathological molecular markers to reveal the feasibility of TCM syndrome differentiation in the diagnosis of CRC. We found that TCM syndrome types were strongly correlated with pathological types, however, the correlation between TCM syndrome types and pathological molecular markers was weak.

It was obvious to see that deficiency syndromes, including QSSD, DSKY, and DBQB, accounted for higher proportion than excess syndrome (ADH) in CRC. Several studies have showed that deficiency syndrome is closely associated with cancers, such as non-small cell lung cancer [27], nasopharyngeal cancer [28] and pancreatic cancer [29]. Moreover, spleen deficiency occupies an important position in the TCM syndrome of CRC [30].

<table>
<thead>
<tr>
<th>TCM syndrome type</th>
<th>p53</th>
<th>CD44</th>
<th>p53A</th>
<th>Bcl-2</th>
<th>nm23</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH</td>
<td>3 (8.8%)</td>
<td>5 (13.5%)</td>
<td>1 (2.3%)</td>
<td>2 (4.9%)</td>
<td>6 (12.0%)</td>
</tr>
<tr>
<td>QSSD</td>
<td>23 (67.6%)</td>
<td>22 (59.5%)</td>
<td>31 (70.5%)</td>
<td>29 (70.7%)</td>
<td>33 (66.0%)</td>
</tr>
<tr>
<td>DSKY</td>
<td>4 (11.8%)</td>
<td>2 (5.4%)</td>
<td>4 (9.1%)</td>
<td>3 (7.3%)</td>
<td>4 (8.0%)</td>
</tr>
<tr>
<td>DLKY</td>
<td>4 (11.8%)</td>
<td>8 (21.6%)</td>
<td>8 (18.2%)</td>
<td>7 (17.1%)</td>
<td>7 (14.0%)</td>
</tr>
</tbody>
</table>

ADH: accumulated dampness-heat type; QSSD: qi stagnation due to spleen deficiency type; DSKY: deficiency of spleen and kidney yang type; DLKY: deficiency of liver and kidney yin type.

Fig. 1. Correspondence analysis between TCM syndrome types and pathological types.

Note: (1) Independences of row and column variables were examined by chi-square test of this contingency table. The chi-square value was 33.456, P value was 0.001. The results showed that correspondence analysis could be conducted.

(2) ADH: accumulated dampness-heat type; QSSD: qi stagnation due to spleen deficiency type; DSKY: deficiency of spleen and kidney yang type; DLKY: deficiency of liver and kidney yin type; DBQB: deficiency of both qi and blood type.

Fig. 2. Correspondence analysis between TCM syndrome types and molecular markers.

Note: (1) Independences of row and column variables were examined by chi-square test of this contingency table. The chi-square value was 7.344, P value was 0.834. The result showed that correspondence analysis could not be conducted.

(2) ADH: accumulated dampness-heat type; QSSD: qi stagnation due to spleen deficiency type; DSKY: deficiency of spleen and kidney yang type; DLKY: deficiency of liver and kidney yin type.
corresponded to our results that the sum of QSSD and DSKY, which is associated with spleen deficiency, accounted for more than 50% of all cases. Thus, deficiency syndromes may be main characteristics of CRC, which could be used for the diagnosis of CRC.

Correspondence analysis showed that TCM syndrome types were strongly correlated with pathological types. More than 70% of adenocarcinomas were associated with QSSD and DLKY. Similar situation have also occurred in other cancers, such as lung cancer. Previous evidence indicated that deficiency of Qi, and deficiency of Yin are main syndromes of TCM in adenocarcinoma of non-small cell lung cancer [18]. TCM syndrome differentiation has relationship with histopathology of peripheral pulmonary carcinoma [31]. Moreover, for the diseases of digestive tract, TCM syndrome also correlated with histopathology. Patients with chronic superficial gastritis belong to damp heat in the spleen and the stomach syndrome, stomach yin deficiency syndrome has positive correlation with intestine metaplasia [19]. Furthermore, recent report shows that TCM differentiations of syndromes have relationship with histopathology in moderate-late stage CRC [32]. However, the corresponded syndromes to histopathology of CRC are distinguishing from our result. This may be caused by the different stages of CRC patients used in the two studies. Therefore, TCM syndromes may be strongly related with histopathology of CRC, however, the exact TCM syndrome types corresponding with histopathology in different stages of CRC still need to be confirmed.

Nevertheless, our results showed that the correlation between TCM syndrome types and pathological molecular markers was not obvious. This is inconsistent with other studies, which indicated that the expression of CD44, P53 is related to TCM syndrome differentiation of CRC [33,34]. Nm25 has relationship with element of different syndromes [35]. In addition, Bcl-2 has been reported to be associated with TCM syndrome differentiation in gastric cancer [36]. The possible reason may be the small sample size in our study. Thus, the relation between TCM syndrome types and pathological molecular markers still need to be investigated.

There are some inherent limitations to our study. Firstly, for the inter-examiner reliability between two expert TCM physicians, formal statistics were not performed. Secondly, the sample size may not have been large enough to investigate the relationship between TCM syndrome types and pathological molecular markers, further study with larger sample size should be conducted. Thirdly, the present study was single-center trial and most of the patients included in the present study were recruited from around Shanghai in eastern China, which might cause geographic bias. Nonetheless, this study also could provide a reference for investigating the relationship between TCM syndrome differentiation and histopathology of CRC.

In conclusion, deficiency syndromes are the main characteristics of CRC. TCM syndrome differentiation is strongly correlated with histopathology of CRC. This finding may lead to the development of TCM syndrome differentiation as a supplement in the diagnosis of CRC. However, the relationship between TCM syndrome types and pathological molecular markers need to be further verified.

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1 Mechanism Study of Recurrence and Metastasis Reduction of Transplanted Colorectal Cancer in Nude Mice Treated with Changyijian Decoction No. 12ZZ123.

2 Effect of Bushen-Jianpi Decoction on Postoperative Recurrence and Vascularogenic Mimicry Formation after Recurrence in Liver Cancer Tissues of Nude Mice with Transplanted Liver Cancer No. 2012J003A.

3 The Study of Electric Acupuncture in Preventing the Peripheral Neuritis Induced by Chemotherapy and Promoting the Immune Function in Breast Cancer Patients From: Korea Comprehensive and Integrative Medicine Institute.

4 Mechanism Study of Fuzheng Jianpi Decoction on Tumor Metastasis Inhibition by Protecting Intestinal Barrier and Regulating P38/AKT Signaling Pathway Expression in Colorectal Tumor Microenvironment No. 15ZR1438700.

Conflict of interest

None

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